Impact of Two Remineralizing Agents on White Spot Lesions

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Research article

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Abstract

Aim of the study: This study evaluated the impact of two remineralizing agents containing casein phosphopeptide–amorphous calcium phosphate (CPP–ACP), and Tricalcium phosphate (TCP) on white spot lesions.

Materials and Methods: A total of ninety freshly extracted upper premolars were divided in three equal groups (30 each). Group A: Control group. Group B: Varnish containing CPP–ACP. Group C: Varnish containing TCP. The teeth were subjected to Diagnodent test after inducing white spot lesions to assess any surface changes present on the teeth. The readings were repeated post demineralization and post-remineralization.

Results: Statistically significant difference was present in the mean values between the tested groups. CPP-ACP group recorded higher mean value at demineralization when compared to TCP group. Statistically significant difference ($p < 0.0001$) was present between the baseline and the demineralization values. CPP-ACP group presented higher remineralization results when compared to TCP group. T-test proved a significant difference between the tested groups.

Conclusion: CPP-ACP containing varnish had higher remineralizing effect when compared to TCP containing varnish.

Clinical Significance: Recently, the focus of restorative dentistry has been directed toward a conservative approach. Prevention and non-restorative treatment of initial enamel caries is the main concern. Remineralization procedures are the most preferred way of regeneration of lost tooth structure.

Introduction

Dental caries is one of the major dental challenging problems maybe more prominent than other infectious diseases such as hypertension and heart infections. ⁽¹⁾ It is easily detectable and reversible at an early stage. Once the incipient lesion proceeds to cavitation, the condition becomes irreversible. That is why it is important to prevent the progression of caries at early stages, instead of developing treatment procedures to treat its advanced stages. ⁽²⁾ White spot lesions are the earliest macroscopic evidence of enamel caries. Early stages of tooth demineralization are reversed by saliva, which contains fluoride, calcium, and phosphate ions, buffering agents, and other substances. ⁽³⁾ After orthodontic treatment, studies proved that white spot lesions can develop within 1 month and it varies from 2%- 96% ⁽⁴⁾ and fluoride is the most commonly agent used to treat these white spot lesions such as topical fluorides ⁽⁵⁾ but the major inadequacy of the toothpastes, mouth rinses, and topical applications is their limited ability to remineralize enamel by the low concentration of calcium and phosphate ions available in saliva. ⁽⁶⁾ New remineralization materials have been launched out, among which the wellknown is the use of fluoride. ⁽⁷⁾ A few of them are bioactive glass, casein phosphopeptide–amorphous calcium phosphate (CPP–ACP), hydroxyapatite with fluoride. ⁽⁸⁾ Fluoride varnish is a standard remineralizing agent created to

drag out the contact time between fluoride and the tooth surface acting as a moderate discharging store of fluoride. Including calcium phosphate salts, such as TCP to the varnish, may improve the mineralization of dentin. To upgrade the impact of the varnish, calcium and phosphate have been included.⁽⁹⁾ Remineralization and anticariogenic mechanism of CPP–ACP includes the joining of nanocomplexes into dental plaque and onto the tooth surface, which in this manner acts as a calcium and phosphate reservoir. Several methods have been used to assess the degree of remineralization, including DIAGNOdent, quantitative light-induced fluorescence (QLF), scanning electron microscopy, polarized light microscope, x-ray spectrophotometer, standard photography, micro-computed tomography and transverse micro-radiography.⁽¹⁰⁾ Diagnodent is a laser device measures fluorescence, early demineralization with good to excellent sensitivity and improves the demonstrative precision of location of non-cavitated carious lesions.⁽¹⁾ This study will use Diagnodent to evaluate the impact of two remineralizing agents containing casein phosphopeptide amorphous calcium phosphate (CPP ACP), and Tricalcium phosphate (TCP) on white spot lesions.

Material And Methods

A total of ninety freshly extracted upper premolars were used in this study which were extracted for orthodontic purposes after obtaining written informed consent from the patients. Ethics approval was obtained from the Research Ethical Committee, Al-Farabi Private Colleges in Jeddah-KSA (Approval No20-03/6). The sample size calculations were performed using G Power v3.1.3 software (University of Düsseldorf; Düsseldorf, Germany). A power analysis showed that a sample size of 30 specimens per group was found to meet the constraints of $\alpha = 0.05$ and power = 0.90. Teeth were washed under water and scaled using a sharp hand scaler to remove any plaque, calculus, or soft tissue remnants then stored in normal saline at room temperature (approximate 25°C) until used. Then, teeth sectioned vertically, mesiodistally into buccal and lingual halves by cutting machine (E96, USA) using a diamond disk (EDENTA Golden S.A.W, Swiss made). The buccal parts of the teeth were dried and painted with two layers of nail varnish uncovering a window of 2 × 2 mm on the center of the buccal surface of the teeth and left to dry. The teeth were divided into three groups according to the material used (30 each). Group A: Control group: Samples in artificial saliva. Group B: Varnish containing CPP–ACP (MI varnish GC, USA). Group B: Varnish containing TCP (3M, ESPE, M Vanish 5% Sodium Fluoride White Varnish with TCP, USA). Diagnodent (Kavo, Biberach, Germany) was used to examine if there are any surface changes on the labial window. Only samples with 3 and 7 values on the digital display were used in this study as Diagnodent score between 3 and 7 indicates normal enamel.⁽¹¹⁾ The baseline values of all groups were recorded. Samples were immersed in three glass containers with 12 ml of demineralizing solution according to each group for 9 days at room temperature (approximate 25°C)⁽¹²⁾ to induce artificial caries like lesion. The demineralizing solution had the following composition: $\text{CaCl}_2 = 2.2 \text{ mM}$, $\text{NaH}_2\text{PO}_4 = 2.2 \text{ mM}$, Lactic acid = 0.05 M, Fluoride = 0.2pp, adjusted with 50% NaOH to a pH 4.5.⁽¹³⁾ The pH of the demineralizing solution was checked during and after preparation of solution using digital pH meter. The solution was replaced every 3 days.⁽¹⁴⁾ Then the samples washed with deionized water and dried with air

syringe. Group (A): Control group teeth were placed in artificial saliva to prevent dehydration.⁽²⁾ Group (B): Teeth were coated with varnish containing CPP-ACP using microbrush for 4 minutes every 24 hours and placed back in the artificial saliva solution without rinsing for 7 days. Group (C): Teeth were coated with varnish containing TCP in the same way as group (B). The composition of artificial saliva contains: (0.65 g/l potassium chloride, 0.058 g/l magnesium chloride, 0.165 g/l calcium chloride, 0.804 g/l dipotassium hydrogen phosphate, 0.365 g/l potassium dihydrogen phosphate, 2 g/l sodium carboxymethyl cellulose and distilled water) ⁽¹⁵⁾Diagnodentwith probe tip B was used to measure the values. The device was calibrated against a porcelain standard before reading and after testing each 5 teeth. The probe tip B was kept perpendicular to the tooth surface. The values were recorded, tabulated, and statistically analyzed using one-way ANOVA and Tukey's multiple comparison tests. All data were analyzed by SPSS software (SPSS 16.0, Chicago, IL, USA)

Results

Statistically significant difference was present in the mean values between the tested groups. CPP-ACP group (14.73) recorded higher mean value at demineralization when compared to TCP group (13.87). Statistically significant difference ($p < 0.0001$) were present between the baseline and the demineralization values. CPP-ACP group (2.67) presented higher remineralization results when compared to TCP group (2.07). T-test proved a significant difference between the tested groups ($p = 0.019$) (Table 1).

Table (1): Comparison between Two Varnishes

	CPP-ACP	TCP	Total	P Value
Control (A)	3.60±0.507	3.40±0.737	3.50±0.630	0.394
After Demineralization (B)	14.73±0.884	13.87±0.516	14.30±0.837	0.003*
After Remineralization (C)	2.67±0.817	2.07±0.458	2.37±0.718	0.019*

$p < 0.05$ —significant

Discussion

Diagnodent shows higher sensitivity and accuracy in enamel caries detection when compared with other conventional methods (visual and tactile examination and bitewing radiographs)⁽¹⁶⁾ It was developed by Lussi in 2004, a value of 5–25 indicates early lesions, 26–35 indicates early dentin caries, and over 35 indicates deep dentin caries but it is most important disadvantage that it can give false positive results in the presence of plaque and calculus, or soft tissue remnants, therefore the teeth were cleaned properly in this study. ⁽¹⁷⁾ Two commercially available remineralizing agents were used: varnish containing CPP–ACP (MI varnish, GC, USA) and varnish containing TCP (3M, ESPE, M Varnish TM 5% Sodium Fluoride White Varnish with TCP, USA). CPP-ACP is a bioactive agent with a base of milk products able to bind calcium and phosphate ions to stabilize calcium phosphate in solution and to increase the level of

calcium phosphate in dental plaque⁽¹⁸⁾ It was used for the first time in 2009 to treat white spot lesions⁽¹⁹⁾ and many studies proved it is positive effect in prevention of secondary caries. Karlinsey et al.,⁽²⁰⁾ showed that adding calcium phosphate salts, such as tri-calcium phosphate (TCP), to the varnish may improve the mineralization of bovine dentin, but few studies have addressed the effect of tri-calcium phosphate on teeth. These two varnishes have different mechanism of action, but they both proved enhancement in the remineralization process. Remineralization is defined as the redeposition of lost minerals in enamel, and this term is used as a synonymous of repair or rehardening of enamel.⁽²¹⁾ CCP-ACP is a source of the calcium and phosphate that inhibits demineralization and/or enhances remineralization. Prevention of demineralization is possible by the presence of casein which can buffer plaque acid either straightforwardly or through bacterial catabolism. It has the ability to discharge amino acids which accept protons and act as buffers.⁽⁸⁾ Newly remineralization and anticariogenic mechanism of CPP-ACP includes the incorporation of nanocomplexes in the dental plaque and on the tooth surface and acts as a calcium and phosphate store, in addition to its high safety level.⁽²²⁾ The synergistic effect of CPP-ACP and fluoride in reducing the white spot lesions is related to the formation of CPP-stabilized amorphous calcium fluoride phosphate which increased the concentration of bioavailable calcium and phosphate particles.⁽²³⁾ Another studies proved that CPP reduce the adherence and functioning of cariogenic streptococcus bacteria in the oral cavity.⁽²⁴⁾ In this study, statistically significant difference presented in the mean values between the tested groups. The mean value showed high demineralization results for CPP-ACP group (14.73) when compared to TCP group (13.87). Statistically significant difference ($p < 0.0001$) was also presented between the tested groups at the baseline and the demineralization value. Best results in remineralization was observed in CPP-ACP group (2.67) when compared to TCP group (2.07). Significant difference was observed between the tested groups ($p = 0.019$). Kamath et al.,⁽²⁵⁾ in their study on the initial carious lesions evaluated the effect of different remineralizing materials includes tricalcium phosphate TCP, CPP-ACFP and nanohydroxyapatite and all these materials had a remineralization effect. TCP significant remineralizing effect is clarified by the high levels of calcium and phosphorus ions in addition to the presence of fluoride ions in the saliva which is considered as an appropriate remineralization solution which encourage the remineralization of the initial carious lesions. The results agreed with Bajaj et al.,⁽²⁶⁾ who treated initial carious lesions with functional TCP milled with sodium lauryl sulfate. Enamel remineralization of different calcium-phosphate and fluoride delivery systems were assessed with Vyavhare et al.,⁽²⁷⁾ who compared the remineralization potential of fluoride, nano-hydroxyapatite, and CPP-ACP and they stated that tested materials had high remineralization potential of fluoride. Also, Ebrahimi et al.,⁽²⁸⁾ agreed with the results of multiple studies who proved significant reduction of white spot lesions after the use of products containing CPP-ACP or CPP-ACPF. The results of this study agreed with Damyanova et al.,⁽²⁹⁾ who used Clinpro white varnish and TCP and stated that it is effective lessening demineralization within the subsurface layer and in improving the remineralization of surface and subsurface enamel layer. Majithia et al.,⁽³⁰⁾ observed that varnish with CPP-ACP with fluoride (MI Varnish) had better results in remineralization when compared to the other materials. Cochrane et al.,⁽³¹⁾ observed a fast discharge of calcium and inorganic phosphate particles under neutral condition which is reliable with the bioavailable nature of CPP-ACP contained

within the varnish and that was related to high water solubility of CPP-ACP. Savas et al.,⁽³²⁾ recommended the use of CPP ACP containing fluoride varnish (MI Varnish) in the dental clinics as it improves the remineralization of incipient carious lesions after a single application. On the other hand, multiple remineralization researches done by Karabekiroglu et al.,⁽³³⁾ and Robertson et al.,⁽³⁴⁾ stated that the efficiency of casein phosphopeptide amorphous calcium phosphate is unreliable in reducing the white spot lesions. This was explained by El zankalouny et al.,⁽¹⁵⁾ who stated that the impact of CPP-ACP localizes the amorphous calcium phosphate by CPP onto the tooth surface, which makes a hypersaturation state regarding the tooth mineral. In another study, it was proved that CPP-ACP alone without the presence of fluoride could not prompt the formation of fluorapatite in the deep white spot lesions.⁽³⁵⁾ However, within the limitation of this study, as Diagnodent is not as precise as a scanning electron microscopy, further clinical trials are recommended to evaluate the effect of these agents and to speed up the repair process of the white spot lesions for better esthetic.

Conclusion

CPP-ACP containing varnish had higher remineralizing effect when compared to TCP containing varnish.

Clinical Significance:

Recently, the focus of restorative dentistry has been directed toward a conservative approach. Prevention and non-restorative treatment of initial enamel caries is the main concern. Remineralization procedures are the most preferred way of regeneration of lost tooth structure.

Abbreviations

TCP: Tricalcium phosphate

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate

°C: Degree Celsius or degree centigrade

pH: Power of hydrogen

SPSS: Statistical Package for the Social Sciences

Declarations

Ethics approval and consent to participate:

Ethics approval was obtained from the Research Ethical Committee, Al-Farabi Private Colleges in Jeddah (Approval No20-03/6) and written informed consent was obtained from the donors.

Consent to publish:

Not applicable

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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The authors declare that they have no competing interests.

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EA designed the study and performed the experiments and collected the data and wrote and revise the manuscript.

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